

Amendments to the Specification:

At page 1, line 1, replace the title with the following replacement title as follows:

~~Closure of bacterial ghosts by means of bioaffinity interactions~~ Sealing closure of bacterial ghosts by means of bioaffinity interactions

At page 11, line 23, please amend the fifth paragraph as follows:

Biotinylated ghosts are obtained by expressing an E-ivb fusion protein (ivb = *in-vivo* biotinylation sequence). Biotinylated membrane vesicles are obtained\* by expressing the E'-ivb fusion gene and then preparing vesicles. Streptavidin is used as the bridge to the biotinylated E protein.

At page 13, line <sup>5</sup>19, please amend the first paragraph as follows:

Standard methods (Sambrook and Russel, MolecularCloning, A Laboratory Manual (2001) 3rd Ed., Cold Spring Harbor Laboratory Press) were used to clone the sequences encoding a minimal peptide substrate for *in-vivo* biotinylation (ivb) (Beckett et al., Protein Sci. 8 (1999), 921-929; Schatz, Biotechnol. 11 (1993), 1138-1143) and an  $\alpha$ -helical peptide ( $\alpha$  spacer; amino acid sequence GGAAAKAAAKAAAKGG (SEQ ID NO. 1); Chakrabarty et al., Biochemistry 32 (1993), 5560-5565; Vila et al., PNAS USA 89 (1992), 7821-7825 and Padmanabhan and Baldwin, protein Sci. 3 (1994), 1992-1997) into the vector pKSEL5-2 (Szostak et al., J. Biotechnol. 44 (1996), 161-170) which, after restriction cleavage with BamHI and SacI, encodes the E' anchor (see ~~figure 4B~~ Figure 4B).

At page 14, <sup>line 7</sup>lines 12 and 16, please amend the second paragraph as follows:

The expression plasmid pL' $\alpha$ ivb, which expresses a fusion protein comprising the L' membrane anchor of the phage MS2 (56 C-terminal codons of the lysis protein L) fused to the  $\alpha$  spacer and the ivb sequence, with the ivb sequence being arranged at the N terminus of the fusion protein (~~figure 1B~~ Figure 1B), was prepared in an analogous manner. The plasmids pL' $\alpha$ ivb-IPTG and pL' $\alpha$ ivb-Ara, which express the L' $\alpha$ ivb fusion protein under the control of promoters which can be regulated by IPTG and, respectively, arabinose (~~figures 1C~~ Figures 1C and 1D) were also prepared from pL' $\alpha$ ivb.

At page 14, line 19, please amend the third paragraph as follows:

The plasmid pSL878ivb (~~figure 1B~~ Figure 1E) was prepared from the plasmid pSL878 (Hovorka et al., FEMS Microbiol. Lett. 172 (1999), 187-196; Kuen et al., Mol. Microbiol. 19 (1995), 495-503) by inserting the *in-vivo* biotinylation sequence ivb at position 878 in the S layer sbsA gene.

At page 14, line 25, please amend the third paragraph as follows:

The plasmid pFNEivb2, shown in ~~figure 1F~~ Figure 1F, was prepared as described in Mayrhofer (2003, see above).

At page 17, line <sup>1</sup>~~13~~, please amend the first paragraph as follows:

... bacterial ghosts, both the lysis protein and the membrane vesicles were altered such that it became possible to target the vesicles to the lysis tunnel by way of a specific receptor binding. For this, a DNA sequence encoding an *in-vivo* biotinylation signal (ivb) was fused to the 3' end of the E lysis gene. As a result, the E protein is already biotinylated in the cell, with this not, however, impairing the lysis properties. Ghosts which have been prepared in this way exhibit a lysis tunnel which is labeled with a large number of biotin molecules and is therefore a preferred binding partner for membrane vesicles which exhibit membrane-anchored streptavidin molecules (~~figure 2~~ Figure 2). It was possible to demonstrate that calcein was efficiently packaged in ghosts.

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At page 17, line <sup>19</sup>~~24~~, please amend the third paragraph as follows:

Expression vectors were constructed in order to be able to prepare vesicles which were biotinylated like the lysis protein and are able, after streptavidin has been added as linking agent, to dock specifically with the lysis tunnel by way of biotin-streptavidin-biotin interaction (~~figure 3~~ Figure 3).

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At page 17, line <sup>20</sup>~~27~~, please amend the fourth paragraph as follows:

Plasmids for expressing the ivb sequence together with membrane anchors (L'-ivb, ~~figure 4A~~ Figure 4A; E'-ivb, ~~figure 4B~~ Figure 4B) were prepared, with these plasmids also permitting differing expression control. The inducible promoters which were selected were the arabinose,

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lactose and left lambda pL promoters. In addition, a short spacer ( $\alpha$  spacer) was inserted between the *in-vitro* biotinylation sequence and the membrane anchor in order to ensure better accessibility of the biotin molecule.

At page 18, line <sup>9</sup>12, please amend the second paragraph as follows:

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In subsequent work, E' -ivb-carrying membrane vesicles were used in closure experiments. The positioning of the modified membrane vesicles over the ghosts is very clearly visible on microscope photographs (~~figure 5~~ Figure 5). In every case, the vesicles were observed at regions of the ghosts where the E lysis tunnels were also to be observed. It was not possible to find such vesicle accumulation in control assays using unmodified membrane vesicles.

At page 18, line <sup>18</sup>21, please amend the third paragraph as follows:

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When ghosts were incubated with modified membrane vesicles which were additionally loaded with calcein, it was also possible to observe that the fluorescence was translocated from the vesicle to the ghost (~~figure 6~~ Figure 6). This can be explained by fusion of the vesicle with the membrane system of the ghost envelope and an influx which is associated with this, of the calcein into the ghosts.